

Applied research: the ironic and the specific

Oswald Avery's immunological studies on bacterial pneumonia led to modern molecular biology, but were overtaken clinically by chemotherapy. Today the disease is still a serious one, obscured by our over-reliance on penicillin, and there is renewed interest in an immunological approach

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It is often argued that pure research more than pays for itself because some of it can be applied. Fewer examples are quoted that show how applied research has yielded dividends in pure science. In medicine, it is not uncommon to find that research over many years on a specific disease has led to important discoveries in general, while applications to alleviate the disease have seemed fruitless. The story of lobar pneumonia caused by pneumococci (in contrast to virus pneumonia) is such a history.

One of the main goals of the Rockefeller Hospital in New York for its first 30 years was to find ways to treat and prevent pneumonia. During this time, the driving force was a remarkable man, Oswald Avery, who had a series of brilliant collaborators. Avery obtained his medical degree in 1904 but from 1912 to 1946 his work was characterised by its emphasis on biochemical specificity—an emphasis far in advance of its time. He pursued the pneumococcus, using this bacterium both as a tool for looking at the fundamental processes of life and as a pathogen to be conquered through knowledge of these processes. He pioneered new approaches to treatment, only to see pneumonia conquered by new drugs.

In the 1920s pneumonia caused almost one eighth of all deaths in the US and affected persons of all ages and classes. It had a high mortality rate and there was no effective treatment. The first attempts had been based on the use of "immune serum" containing antibodies against the bacterium—a treatment already successfully used against diphtheria. This did not work for pneumonia, however, because there were several different types of pneumococcus which were antigenically separate. On joining the Rockefeller in 1913, Avery developed a specific serum therapy and spent several years in typing pneumococci (discriminating the various antigenic types). For 25 years identification of the different types was critical for treatment, and the problem of the specificity of the types dominated research. Avery found that a soluble substance, specific for the particular type in culture, was present in the culture medium in which the pneumococcus was grown. He used the presence of these specific substances in the urine of patients as a diagnostic test.

Although a specific serum was available from 1913 for Type I pneumonia, effective sera against the other types became available only over the next 25 years. At first they were produced by inoculating the pneumococci into horses, and the resulting sera were liable to produce hypersensitivity reactions. In 1929, the horse was replaced by the rabbit, which produced a more potent antiserum in a shorter time. Later, it was found

that the immunoglobulin fraction could be more easily separated from the non-specific proteins. The best results were still obtained with Type I therapy, and even so the case-fatality rate was only halved. The use of these sera was not widespread, partly because trained technicians had to type the pneumococcus from each patient, with an attendant delay. Some of the clinical trials were not very convincing and the treatment itself was not easy. Eventually, however, the Massachusetts health authorities launched a comprehensive control programme in 1931 and New York State followed in 1936. The success of these programmes was followed by federal funding of other state programmes.

Almroth Wright (from St Mary's Hospital, London) had tried prophylactic immunisation unsuccessfully in South Africa in 1911. Knowledge of the different types of pneumococci was necessary to produce successful vaccines and from 1915 whole bacteria or the purified type specific polysaccharides were used. Mice and rabbits can be immunised with almost uniform success but man apparently cannot and the field trials were never convincing.

In his researches into the biochemical specificity of the types of pneumococci Avery had identified the bacterium's polysaccharide capsule as being the type-specific substance. Moreover, while those bacteria with capsules caused pneumonia, those without capsules were not pathogenic and were quickly engulfed by the phagocytes in the bloodstream. With the help of René Dubos, Avery searched for a bacterium which produced an enzyme that would specifically break the polysaccharide linkages. In 1931 they found a bacillus with such an enzyme and showed that it did indeed dissolve the capsule of pneumococci, which were then engulfed by the phagocytes. By injecting mice with the enzyme (which Dubos purified) they found that mice could be protected with only one injection up to 18 hours after infection. The mice could be protected against a million fold increase in the lethal dose of virulent Type III pneumococcus. In 1932 they cured rabbits by using the enzyme and in 1934 cured Java monkeys to which they had given a disease similar to clinical lobar pneumonia in man. There is no record of further experiments or attempts to cure pneumonia in man.

In 1938, all previous work was overshadowed by the dramatic success of a new "wonder" drug, the sulphonamide M and B 693. The first drugs of this family were ineffective but sulphapyridine reduced the case mortality rate of pneumonia by nearly 70 per cent. Moreover it was cheap, easy to administer without delay, and effective against all types of pneumococcus. Pneumonia control



programmes based on typing and the injection of type specific serum were abandoned. In 1945 penicillin was shown to be effective in pneumonia caused by any type of pneumococcus and, because of its low toxicity, became the drug of choice. In his original paper in 1929, Alexander Fleming had shown that of the bacteria he tested the pneumococcus was the most sensitive to penicillin.

Thus all the painstaking research on pneumococci and the organisation of the control programmes were overtaken by unrelated and unforeseen discoveries. Yet the research on pneumococci itself led to other unforeseen and important advances.

Oswald Avery's influence on science was considerable. He investigated the chemical specificity of the substances released into the culture media used to grow pneumococci and found that they were complex polysaccharides. He and Michael Heidelberger showed that, when injected, these substances stimulated antibody formation and were thus antigenic. This was a remarkable discovery, for up to then it had been a paradigm that antigens were proteins. They then isolated nucleoprotein(s) which was species-specific (not type-specific) and which was also antigenic. Forty years later, there has been a revival of interest in such antigens. After Avery and Dubos had found an enzyme which specifically dissolved the pneumococcal capsule, Dubos looked for "microorganisms capable of attacking . . . intact living (bacterial) cells". In 1939 he reported a bactericidal agent isolated from a bacillus, showing that it was not a protein, and that it cured mice injected with pneumococci. He had discovered the antibiotics Gramicidin and Tyrothricin. This was the first major experiment in animals with what we now call the antibiotics.

Fred Griffith was the British expert on the types and typing of pneumococci. In 1928 he reported his classic experiments on the transformation of pneumococcal types. He injected avirulent, capsuleless bacteria into mice, together with killed capsulated bacteria; the mice died and capsulated bacteria of the same types as those injected killed could be recovered from the animals. This was not only remarkable, it was also interesting from a clinical point of view. It was soon confirmed and in the next decade a series of workers in Avery's laboratory showed that transformation could occur in the test-tube. Avery realised not only the significance of this discovery but also the means of investigating it. All his previous work on the purification of enzymes (proteins) and of polysaccharides and the immunological specificity of proteins, polysaccharides, and nucleoproteins provided him with the technical insight necessary

to isolate and identify the material which was specific for the heritable change in transformation. It is ironic that the proof that this was deoxyribonucleic acid (DNA) was chemical and not immunological. Oswald Avery, Colin MacLeod and Maclyn McCarty published their now classical paper on this work in 1944, one year after Avery's retirement at the age of 65.

Avery and Fleming both died in 1955. Their lives had curious and interesting parallels, even though their work had been on different organisms and their way of work was so dissimilar. Avery was specificity-minded before his time; Fleming was a naturalist. Whereas Avery purified everything he touched, Fleming was unable to do so: not only did he lack the necessary background, experience and perhaps temperament but also, he was not in contact with those who could have helped him. Fleming's use of lysozyme to promote phagocytosis was similar to the later use of capsule-dissolving enzyme: the discovery of penicillin was mirrored by that of gramicidin.

Whether, in each case, Avery and Dubos were influenced, consciously or subconsciously, by Fleming's work is difficult to know; certainly they were aware of his discovery of penicillin. On two occasions, Fleming read papers to the Medical Research Club, on lysozyme and on penicillin, only to be heard in silence. Twenty-five years later he was still talking of "that frightful moment" when there were no questions, no interest. When Avery presented his work on DNA to a staff meeting at the Rockefeller, he too was disappointed by the lack of response. Fleming received a Nobel Prize and many other honours but Avery was overlooked for the former although he received the Copley Medal, the highest award of the Royal Society.

Ironically, in the year that penicillin was shown to be so effective against pneumonia, Colin MacLeod tested the first effective vaccine. It consisted of purified capsular polysaccharides. Further trials confirmed its effectiveness and it was licensed, but was little used and was withdrawn from use in the early 1950s. Typing was no longer necessary with penicillin therapy, and the necessary standard antisera are now difficult to obtain and seldom used. Yet pneumococcal pneumonia is still a common illness with a significant mortality; it caused 50 000 deaths in the US in 1965. Over-reliance on the efficiency of penicillin and a decline in the use and knowledge of laboratory diagnostic techniques have resulted in a situation where complacency masks reality. In the US, there are now pilot investigations of the incidence of pneumococcal infections in hospitals and renewed interest in the vaccines.

Whenever a technological innovation, such as the use of antibiotics, renders previous efforts apparently obsolete, much of value is lost. The pathology of pneumococcal pneumonia was never clear: in certain definable groups of the population, infection still carries a significant risk of death—a risk little lessened by antibiotic therapy. Further research into the pathology might be of general interest as well as of practical use.